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Interspecific hybrids from cross incompatible relatives of sweetpotato

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Summary

Hybrids were obtained from *Ipomoea* interspecific crosses through ovule culture. The hybridity of the progeny obtained from *I. triloba* × *I. trifida* and (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) crosses was established by comparisons of floral morphology and analyses of peroxidase and esterase isozymes. The hybrids displayed the inflorescence type and sepal shape and texture of their male parents, while corolla size and anther and nectary color tended to be intermediate to their parents. The isozyme banding patterns of the hybrids contained bands present in the patterns of each of their parents. Pollen grain viability, measured by aceto-carmine stainability, was 44.1%, 92.3% and 82.4%, respectively, for the *I. triloba* × *I. trifida* hybrid and the (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids, H_1 and H_2. A controlled pollination study revealed that the *I. triloba* × *I. trifida*, and the (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids, H_1 and H_2 were partially self fertile with 6%, 70% and 13%, respectively, of the pollinated flowers producing viable seed. Success in backcrossing and sib-mating varied with the cross combination.

Introduction

Sweetpotato (*Ipomoea batatas* L. (Lam)) (2n = 6x = 90), a member of the section Batatas in the family Convolvulaceae, is a widely grown root crop of substantial economic importance. The phylogeny of the cultivated sweetpotato has been of considerable interest but remains obscure (Austin, 1988; Kobayashi, 1984; Nishiyama et al., 1975; Teramura, 1979; Wedderburn, 1967). Austin (1988) suggests that *I. triloba* L. (2n = 2x = 30) and *I. trifida* (H.B.K.) G. Don (2n = 2x = 30) are sweetpotato's closest extant relatives and its probable progenitor species. Hybridizing these two species to study Austin's hypothesis has not been possible because the species belong to different genome groups and are cross incompatible (Nishiyama, 1982). *I. trifida*, like *I. batatas*, has been classified in the B-genome group whereas *I. triloba* belongs to the A-genome group (Jones & Deonier, 1965; Nishiyama et al., 1975).

Genome differentiation and incompatibility among species within the section Batatas has limited the utilization of wild species for sweetpotato improvement (Martin, 1970, 1982; Shiotani et al., 1990; Teramura, 1979). A few B-genome species have been used to improve sweetpotato quality and disease resistance (Hozyo & Kato, 1973; Iwanaga, 1988; Iwanaga et al., 1991). Species in the A-genome group may also be sources of genes for sweetpotato improvement. Thus far, evaluation and interest in species belonging to the A-genome group has been limited due to cross incompatibilities which have restricted their use in sweetpotato breeding (Wedderburn, 1967). A-genome species such as *I. triloba*, which can be found in both very dry and extremely wet habitats (Martin & Jones, 1973), may be a potential source of drought tolerance and resistance to root rots and foliar fungal diseases. Our attempts and attempts by others (A. Jones, personal communication) to introgress genes from A-genome

into sweetpotato using conventional breeding techniques have, thus far, not been successful.

We developed ovule culture methods, for sweetpotato and other *Ipomoea* species, as a possible means of overcoming certain post-zygotic incompatibility barriers (Kobayashi et al., 1993). In a few A- × B-genome crosses we noted calyx swelling followed by premature flower abscission, therefore, we explored the possibility of using ovule culture as an approach to obtain A- × B-genome hybrids. Using ovule culture, we were able to obtain a plant that appeared morphologically distinct from either parent in an *I. triloba* × *I. trifida* cross. Here, we describe this apparent hybrid, present biochemical evidence of its hybridity and present crossing data to demonstrate its fertility. We also describe some hybrid characteristics of two plants, obtained through ovule culture, from another A- × B-genome cross combination. The roles that these interspecific hybrids could have in improving sweetpotato and in elucidating its origin are discussed.

Materials and methods

Seeds of diploid *Ipomoea trifida* (P.I. 540723) and *I. triloba* L. (P.I. 536043) were obtained from R.L. Jarret (USDA, ARS, Southern Regional Plant Introduction Station, Griffin, GA, USA). Shoot cuttings of tetraploids *I. batatas* (Ac. 79.5) and *I. triloba* × *I. lacunosa* (C54-15) were obtained from A. Jones (USDA, ARS, Vegetable Laboratory, Charleston, SC, USA) to test the application of ovule culture to other A- × B-genome combinations. These lines were chosen because Jones (personal communication), after a number of attempts, had not been able to obtain progeny but did obtain a few non-viable seed from crosses between *I. triloba* × *I. lacunosa* and *I. batatas* (4x). Crosses to obtain the interspecific hybrids and evaluate their fertility were made by emasculating flowers on the afternoon prior to anthesis and then pollinating the next morning.

From 478 *I. triloba* P.I. 536043 (A-genome) × *I. trifida* P.I. 540723) (B-genome) pollinations, 17 flowers exhibited calyx swelling and their ovules were cultured using previously described methods (Kobayashi et al., 1993). Four of the ovules contained small (approximately 150 μm) green embryos and three contained small yellow embryos within a dry seed cavity. No embryos were observed in the remaining 10 cultured ovules.

From 21 (*I. triloba* × *I. lacunosa*) C54-15 (A-genome) × *I. batatas* (4x) Ac. 79.5 (B-genome) pollinations, 8 exhibited calyx swelling and their ovules were cultured. Four of the cultured ovules contained green embryos, approximately 500 μm in length, in a convoluted seed cavity while the other four contained no embryo. The ovules appeared to have 2–3 sections or chambers within the seed cavity. A few of the ovules appeared to contain liquid endosperm within one of the chambers of the seed cavity. The liquid endosperm however, was not observed in chambers that contained an embryo and did not appear to be associated with success in culturing the embryos.

After 10 weeks in culture, only one green *I. triloba* × *I. trifida* embryo survived while two of the four green (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) embryos continued to develop into whole plants. Plantlets obtained through ovule culture were transferred to pots (7.6 × 7.6 cm) containing Jiffy Mix soilless potting medium. All plants were grown in Conviron growth chambers under 220 $\mu molm^{-2}s^{-1}$ irradiance at 27° C.

To assess pollen grain viability, the percentage of pollen grains stained by aceto-carmine was obtained at anthesis. For isozyme analysis, extracts were made from stems and leaves of four week old in vitro grown plants. The tissue were ground in buffer (1 g/ml) consisting of 12.1 mg l^{-1} Tris, 1 mg l^{-1} glutathione, 7.5% (w/v) ascorbic acid, 20% (w/v) sucrose with 1% (w/v) bromophenol blue as tracking dye, using ice-cold glass tissue homogenizers. Electrophoresis was carried out using Tris/glycine, pH 8.5, running buffer in a Mini-PROTEAN II vertical slab cell (Biorad, Richmond, CA) at a constant 200 V for 45 minutes. Isozymes were separated on 7.5% (w/v) polyacrylamide Tris-HCl gels. Gels were stained for peroxidase (E.C.1.11.1.7) and esterase (E.C.3.1.1.2) as described by Kobayashi et al. (1987).

Results and discussion

Morphologically, the *I. triloba* × *I. trifida* hybrid we obtained has a vine-like growth habit, cordate, entire leaves and is diploid, root tip chromosome number 2n = 30. It has flowers that develop singularly and have a lavender corolla with purple center (2–3 cm in length), stamens with white filaments and lavender anthers, a light yellow nectary and pilose ovate sepals. The hybrid has the inflorescence type and sepal shape and texture of its male parent and the anther color of

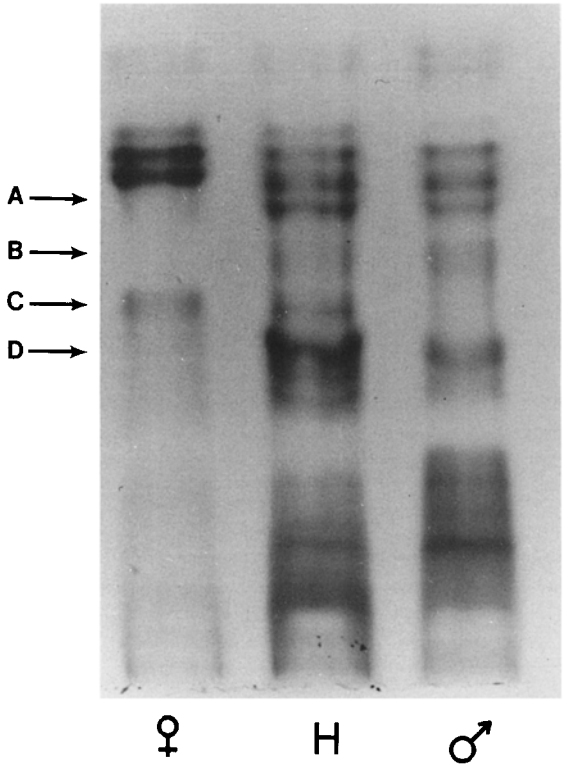

Fig. 1. Peroxidase zymogram of *I. triloba* P.I. 536043 (♀), *I. trifida* P.I. 540723 (♂), and their hybrid (H).

its female parent. The corolla size and nectary color of the hybrid's flowers tends to be intermediate to its parents (Table 1).

The two (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids, designated as H_1 and H_2, have vine-like growth habits, cordate, entire leaves, flowers with lavender to light lavender corollas with purple centers (3.5–4 cm in length) borne in cymose inflorescences and appear to be tetraploid. The flowers of H_1 have lavender anthers, yellow nectaries and glabrous ovate sepals. The flowers of H_2 have white anthers, light yellow to yellow-green nectaries and glabrous ovate sepals. Both hybrids have the inflorescence type and sepal shape and texture of their male parent. The flowers of H_1 have a nectary and anther color that tends to be intermediate to its parents while H_2 flowers have the nectary color of its female parent and the anther color of its male parent (Table 1).

Hybridity was confirmed through the use of peroxidase and esterase isozymes. The peroxidase and esterase (data not shown) banding patterns of the *I. triloba* × *I. trifida* hybrid contained bands present in

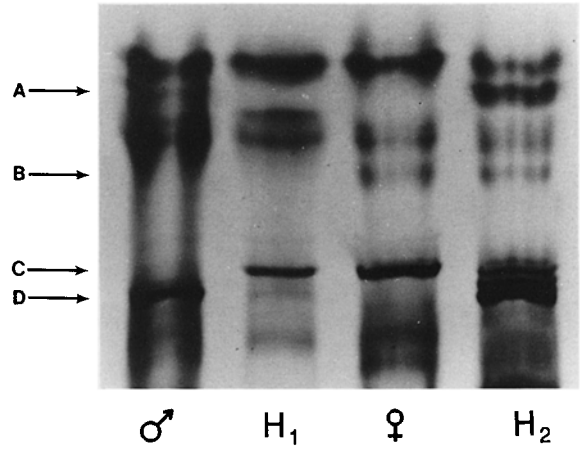

Fig. 2. Esterase zymogram of *I. batatas* (4x) Ac. 79.5 (♂), (*I. triloba* × *I. lacunosa*) C54-15 (♀), and their hybrids (H_1 and H_2).

the patterns of both parents (Fig. 1). Band C is present in the *I. triloba* parent and the hybrid but not in the *I. trifida* parent. Conversely, bands A, B and D are present in the hybrid and the *trifida* parent but not in the *I. triloba* parent.

The hybridity of the *I. triloba* × *I. trifida* plant was clearly established by the peroxidase isozymes, but the hybridity of both (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids, H_1 and H_2, could not be confirmed by peroxidase alone. The hybridity of both (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) progeny was confirmed, however, by esterase isozymes (Fig. 2). The esterase isozyme pattern of H_1 contained band C which was found in the pattern of the *I. triloba* × *I. lacunosa* parent but not the *I. batatas* (4x) parent, and band D found in the *I. batatas* (4x) pattern but not the *I. triloba* × *I. lacunosa* pattern. The pattern of H_2 contains bands A and D found in the pattern of the *I. batatas* (4x) parent and bands B and C found in the pattern of the *I. triloba* × *I. lacunosa* parent. Using peroxidase isozymes, H_1 could be distinguished from both parents but the isozyme pattern of H_2 was not distinguisable from that of the female parent (data not shown).

Based on pollen grain aceto-carmine stainability, the *I. triloba* × *I. trifida* hybrid appeared to have lower pollen grain viability, 44.0%, than either the *I. triloba* (69.1%) or the *I. trifida* (99.7%) parent, as might be expected with interspecific hybrids. One of the (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids also had lower pollen grain viability than either parent while the other appeared to have pollen grain viability equal to its parents (Table 2).

Table 1. Comparison of floral characters of *Ipomoea* species and interspecific hybrids

	Genotype						
	P.I. 536043 (♀)	P.I. 540723 (♂)	Hybrid	C54-15 (♀)	Ac79.5 (♂)	Hybrid H_1	Hybrid H_2
Inflorescence	cymose	solitary	solitary	solitary	cymose	cymose	cymose
Corolla	Funnelform	Funnelform	Funnelform	Funnelform	Funnelform	Funnelform	Funnelform
	lavender	lavender	lavender	purple	lavender	lavender	light lavender
	1.5–2 cm	3–4 cm	2–3 cm	4 cm	4 cm	3.5–4 cm	3.5–4 cm
Anther	lavender	white	lavender	purple	white	lavender	white
Nectary	white	yellow-orange	light yellow	light yellow to yellow-green	yellow-orange	yellow	light yellow to yellow-green
Sepal	7–10 mm	7–10 mm	7–10 mm	12–14 mm	9–10 mm	8–10 mm	13–15 mm
	glabrous	pilose	pilose	hairy	glabrous	glabrous	glabrous
	oblong	ovate	ovate	obovate	ovate	ovate	ovate

Table 2. Percent pollen grain viability based on aceto-carmine stainability

Genotype	Number of pollen grains counted	Percent viability
I. triloba (P.I. 536043)	521	69.1
I. trifida (P.I. 540723)	1210	99.7
I. triloba × *I. trifida*	1495	44.0
I. triloba × *I. lacunosa* (C54-15)	170	87.7
I. littoralis (Ac. 79.5)	935	98.1
I. triloba × *I. lacunosa* × *I. batatas* (4x) (H_1)	1490	92.3
I. triloba × *I. lacunosa* × *I. batatas* (4x) (H_2)	744	82.4

A controlled pollination study revealed that the hybrids were partially self fertile with 6%, 70% and 13%, respectively, of the pollinated flowers producing viable seed from *I. triloba* × *I. trifida* ⊗, (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) H_1 ⊗ and H_2 ⊗. Attempts to backcross the *I. triloba* × *I. trifida* hybrid were successful when the *I. triloba* parent was used as a female (Table 3). A few seeds were recovered in the hybrid × *I. trifida* backcross, but none were viable. The two (*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) hybrids could be readily sib-mated and backcrossed (Table 3).

Increase in the genetic accessibility of related species, through ovule culture or other means, broadens the genetic base that can be used for future sweetpotato germplasm enhancement (Wedderburn, 1967; International Potato Center, 1990). We have demonstrated that hybrids between A- and B-genome species can be fertile. This suggests that A-genome species can be utilized for sweetpotato improvement. The hybrids between A- and B-genome species reported here, might be useful bridges to introgress valuable genes from A-genome species into sweetpotato.

In addition to its possible usefulness for sweetpotato improvement, the *I. triloba* × *I. trifida* hybrid is of particular interest because it may also be useful in elucidating the origin of sweetpotato. There are two popular hypotheses on the origin of sweetpotato. Austin (1988), based on morphology and cluster analysis, has proposed that sweetpotato arose through allopolyploidy and that *I. triloba* × *I. trifida* are its probable ancestors. In contrast, Nishiyama et al. (1975) suggest *I. leucantha* (2n = 2x = 30), *I. littoralis* (2n = 4x = 60) and *I. trifida* (2n = 6x = 90) are the progenitor species of sweetpotato and that sweetpotato evolved through autoploid doubling of the *I. leucantha* genome. Examination of the hypotheses on the evolution of the cultivated sweetpotato could be greatly aided by development of a polyploid series from *I. triloba* × *I. trifida*

Table 3. Successful pollination and seed germination percentages from controlled pollinations of *Ipomoea* species and interspecific hybrids

Cross	No. of Pollinations	Successful Pollinations (%)	Seed Germination (%)
I. triloba (P.I. 536043) × *I. trifida* (P.I. 540723)	478	4[z]	–
I. triloba × *I. lacunosa* (C54-15) × *I. batatas* (4x) (Ac. 79.5)	21	38[z]	–
I. triloba × *I. trifida* ⊗	53	6 (3)[y]	83 (6)[x,w]
(*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) (H_1)⊗	27	70 (47)	100 (20)
(*I. triloba* × *I. lacunosa*) × *I. batatas* (4x) (H_2)⊗	15	13 (2)	100 (2)
H_2 × H_1	19	74 (30)	90 (10)
P.I. 536043 × (*I. triloba* × *I. trifida*)	60	10 (6)	50 (6)
(*I. triloba* × *I. trifida*) × P.I. 536043)	50	0	–
(*I. triloba* × *I. trifida*) × P.I. 540723	45	2 (3)	0 (3)
P.I. 540723 × (*I. triloba* × *I. trifida*)	48	0	–
C54-15 × H_1	3	0	–
H_1 × C54-15	22	14 (3)	67 (3)
H_1 × Ac. 79.5	9	33 (8)	25 (8)
Ac. 79.5 × H_1	2	100 (3)	67 (3)
H_2 × C54-15	23	44 (11)	80 (10)
H_2 × Ac. 79.5	9	75 (16)	0 (12)

[z] Success based on calyx swelling, these were ovule cultured.
[y] Values in parentheses represent the number of seeds obtained.
[x] Values in parentheses represent the number of seeds tested for germination.
[w] Some seeds obtained through natural (un-aided) self pollination.

(Jones & Kobayashi, 1968). Phylogenetic studies with sweetpotato and a 2x, 4x and 6x series of *I. triloba* × *I. trifida* hybrids that involve comparisons of morphological characteristics, crossability (Teramura, 1979) and biochemical and DNA markers (Jarret et al., 1992) should help clarify the relationships among *I. batatas*, *I. triloba* and *I. trifida*.

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